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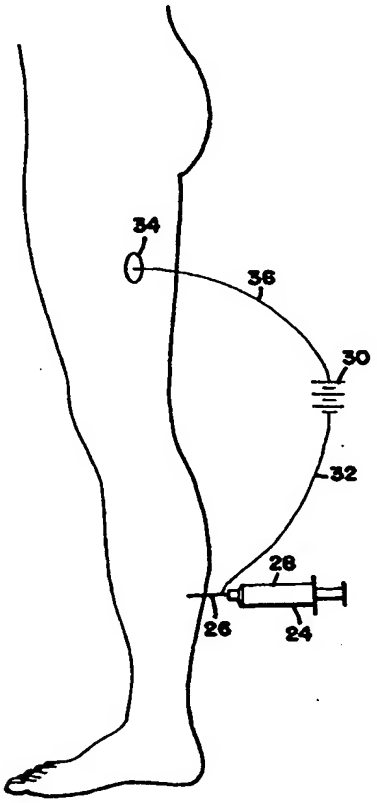
(57) Abstract

An apparatus for delivering genetic material into a target area in a patient's body and mediating cellular expression of genes in the target area. The apparatus comprises a reservoir arrangementconfigured to retain genetic material and supply the genetic material to the target area. A first electrode is configured and arranged to be in electrical communication with the reservoir, and a second electrode is configured to be placed into electrical communication with the target area. A power supply is in electrical communication with the first and second electrodes. The power supply is configured to pass energy between the first and second electrodes at a level of about 0.8 A or below.



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ELECTRICALLY MEDIATED CELLULAR EXPRESSION

Technical Field

The present application relates to electrically mediating cellular expression, and more particularly, to treating cells with low levels of electrical energy and delivering genetic material to the cells.

Background

The past few years has brought great strides in the development of agents that have improved therapeutic and diagnostic applications. For example, scientists and medical researchers are rapidly developing genetic materials that cause cells in the body to behave in a certain way. This new technology has the potential to revolutionize the medical practice and how we treat disease and other maladies that can kill a person or destroy their quality of life.

One of the limitations of such new agents is the mechanism and procedures for delivering the agents, such as genetic material, to cells in a target area. Delivery can occur either in vitro or in vivo. In vitro delivery is the most common technique because it is more efficient than in vivo delivery and it minimizes the risk that the genetic material will be delivered to cells outside of the target area.

During in vitro delivery, tissue having the target cells is removed from the patient's body and grown in a culture in the caregiver's laboratory. The genetic material is delivered to the culture using one of a variety of techniques. These techniques can include chemical techniques such as calcium-phosphate transfection; physical techniques such as electroporation, microinjection, and particle bombardment; and vectors, which are biological vehicles for carrying the genetic material and transmitting the genetic material in the desired cell. A vector can be viral or non-viral. Examples of viral vectors include adenoviruses, certain retroviruses, adeno-associated viruses, viral envelope/capsid-DNA complexes, DNA-protein complexes, as well as other types of viruses. Examples of non-viral vectors include gene plasmids, lipid-based compositions, and liposome-based compositions.

In vitro delivery, however, has several disadvantages. For example, it can be used only with cells that can be removed from the body, genetically treated,

and then grafted back into the body. Many tissues cannot be sufficiently grown in a culture to be treated with genetic material. Other tissues will not survive for a long period of time once grafted back into the patient's body. Furthermore, the patient is simply put at risk during the surgical procedure to remove the tissue, especially tissue that forms vital organs such as the liver.

In vivo delivery overcomes several of the problems with in vitro delivery because the genetic material is delivered to cells that are within the patient's body. No cells are removed from the body. One technique for in vivo delivery involves direct injection of the genetic material into the patient. In this technique, a syringe is used to inject the genetic material into an area of target cells. In theory, the target cells then process the injected genetic material to produce a desired biological reaction. This biological reaction may take one of several forms such as the promotion or inhibition of cell division; the expression of a new protein; and apoptosis, which is programmed cell death.

In practice, however, direct injection also has many shortcomings. One shortcoming is inefficiency in transferring the genetic material into the cells and relatively low level of stable integration of the genetic material into target cells. The transfection efficiency of local delivery by direct injection can be as low as 1% to 2%.

The vectors described above can be used in conjunction with direct injection in an effort to promote the transfer and expression of genetic material in cells. An advantage of vectors is that they exploit characteristics of particular cells, and thus can be used to target particular cells in a complex structure such as the human body. Nevertheless, vectors have many shortcomings for which medical science must find a way to overcome, or at least minimize. For example, many people can have an allergic reaction to viral vectors, which is a significant problem if the patient is already weak and sick. Lipid-based vectors also have shortcomings. They are complex and expensive. They are also difficult to breakdown so that the genetic material can be released in the target cells.

Yet another problem is that vectors are still relatively inefficient. Despite the use of vectors, there is still a large percentage of the target cells that are not transfected with the genetic material. As a result, the caregiver typically has to

deliver a larger amount of the agent in order to transfect an adequate amount of cells. This increased amount drives up the cost of treatment and also increases the risk and severity of unwanted events such as an allergic reaction.

Delivering an increased amount of genetic material also increases the amount of cells transfected in an undesired area of the body. One possible reason for the increased transfection of cells in unwanted areas of the body is that the systemic systems in the human body, such as the vascular or lymph system, will pick up and distribute a certain amount of genetic material delivered to the target site. The more genetic material delivered to the target site, the more genetic material that is picked up by the systemic systems. Another possible reason is that there is simply more genetic material to get washed away from the target area through the interstitial spaces that are between cells.

Electroporation, which was briefly mentioned above, is a technique used to increase the percentage of cells that are transfected. Electroporation involves exposing the target cells to a very high amplitude electric field and literally blasting a hole in the cellular membrane. The hole provides access for the genetic material to reach the cytoplasm of the cell.

Likewise, electroporation also has significant shortcomings. For example, the electrical field strength used is typically in the range from about 2 kV/cm to about 8 kV/cm. Energy at this high of a level can be safely applied for only a short duration and is difficult to use in practice. Therefore, the duration of energy bursts is typically in the range of about 5 μs to about 100 ms. If the duration of energy is too short or the field strength is too low, the cells will not porate. If the duration of electrical field is too long or the field strength is too high, the energy will kill the cell by irreversibly disrupting the target cells and cause thermal damage such as burning, which can be painful for the patient.

Furthermore, electroporation systems draw a high current level and thus the distance between the electrodes used to apply the electrical energy must be very small, typically about 0.3 cm or less. The close spacing of the electrodes is needed to maintain a uniform filed strength. If the spacing becomes too large, the cells close to the electrodes will be subject to energy levels that are too high and be

killed while cells farther away from the electrodes will be exposed to energy levels that are too low and be under porated.

Therefore, there is a need for an improved apparatus and method for enhancing the process of cellular uptake of genetic material and cellular expression. There is a related need for an apparatus and method that does not use high levels of electrical energy. There is another related need for an apparatus and method that provides an increased percentage of the target cells that uptake and express the genetic material. There is also a need for an apparatus and method of enhancing cellular uptake of genetic material and cellular expression that is cost effective, reduces the risk of an allergic reaction, and reduces the risk of genetic expression by non-targeted cells.

Summary

One embodiment of the present invention is directed to a method for delivering genetic material into a plurality of target cells. The method comprises the steps of: delivering genetic material to a position proximal the plurality of target cells; and subjecting the plurality of target cells to electrical energy at a level of about 0.8 A or below, thereby mediating cellular expression.

Another embodiment of the present invention is directed to an apparatus for delivering genetic material into a target area in a patient's body and mediating cellular expression of genes in the target area. The apparatus comprises a reservoir arrangement configured to retain genetic material and supply the genetic material to the target area. A first electrode is configured and arranged to be in electrical communication with the reservoir, and a second electrode is configured to be placed into electrical communication with the target area. A power supply is in electrical communication with the first and second electrodes. The power supply is configured to pass energy between the first and second electrodes at a level of about 0.8 A or below.

Yet another embodiment of the present invention is directed to an apparatus for delivering genetic material into a target area in a patient's body and mediating cellular expression of genes in the target area. The apparatus comprises means for retaining a supply of genetic material, means for transporting genetic material from the means for retaining a supply of genetic material to the target area,

and means for supplying electrical energy of about 20 mA or below to the target area thereby enhancing uptake of the genetic material into cells in the target area.

Description of the Drawings

Figures 1A-1D illustrate the cellular uptake of genetic material, which is enhanced by the present invention;

Figure 2 illustrates cellular uptake by electroporation; and

Figure 3 illustrates an apparatus for delivering genetic material and enhancing the cellular uptake of the genetic material.

Detailed Description

Various embodiments of the present invention will be described in detail with reference to the drawings, wherein like reference numerals represent like parts and assemblies throughout the several views. Reference to the various embodiments does not limit the scope of the invention, which is limited only by the scope of the claims attached hereto.

In general, the present invention is directed to apparatus for, and methods of, using low-levels of electrical energy to enhance the mediation of cellular expression, the process of which includes the cellular uptake of genetic material. The apparatus and method applies a low-level of electrical energy to an area of target cells and delivers genetic material to that target area. The application of the electrical energy can occur either before, during, or after delivery of the genetic material to the target area. Furthermore, the present invention can be utilized in both in vitro and in vivo delivery of genetic material.

Genetic material includes any type of genetic material including RNA and DNA. Furthermore, the genetic material can be in a variety of forms such as naked DNA, naked RNA, anitsense oligonucleotides, ribozymes, gene plasmids, adenoviruses, certain retroviruses, adeno-associated viruses, viral envelop/capsid-DNA complexes, DNA-protein complexes, lipid-based compositions. liposome-based compositions, DNA in complexes with natural or synthetic polymers, and other viral and non-viral vectors.

The process of cellular transfection of genetic material is illustrated in Figures 1A-1D. As shown in these figures, a cell 10 has a nucleus 12, which is surrounded by a nuclear membrane 14. A cellular membrane 16 surrounds the

nuclear membrane 14, and cytoplasm 18 is in the space between the cellular membrane 16 and the nuclear membrane 14. The cytoplasm 18 and nucleus 12 are formed from a continuous aqueous solution that has suspended structures and inclusions.

In the process of cellular uptake, genetic material 20 is initially positioned in the extra-cellular space in close proximity to the cellular membrane 16. The cellular membrane 16 then envelopes the genetic material 20 through a biological mechanism that may involve interaction with a specific membrane receptor. The genetic material then becomes ingested into the cytoplasm 18 as shown in the sequence of Figures 1B and 1C. This process continues until the genetic material 20 is transferred into the cell nucleus 12 as shown in Figure 1D. The genetic material 20 then causes or stimulates a biological reaction, such as upregulation or down regulation of a gene, production of RNA, programmed cell death, or the production and expression of a new protein. Alternatively, some genetic material 20 is ingested through the cellular membrane 16 into the cytoplasm 18. The genetic material 20 remains in the cytoplasm 18 where it results in the desired biological reaction.

There are several possible biological mechanisms that can cause a cell to ingest genetic material. One example is lipid-mediated transfection in which the genetic material is encapsulated in a liposome that fuses with the cellular membrane of the target cell. Another example is receptor-mediated transfection in which the genetic material is taken up by a receptor in the cell membrane.

The present invention has several advantages. For example, the caregiver can apply the electrical energy for extended periods of time with minimal risk of killing the target cells, causing thermal damage to tissue such as burning, and causing pain for the patient. A related advantage is that the tolerance of the energy ranges and pulse widths is not as critical. Having too great a pulse width or to high of an energy level will not result irreversibly disrupt the exposed cells, cause thermal damage, or burn the patient. As a result, the potential danger of improperly calibrating or setting the equipment is greatly reduced.

Another advantage of using lower voltage levels is that the spacing of the electrodes is not as critical. As a result, the electrodes can be separated by a

distance much greater than permitted by electroporation without the concern that some cells close to the electrodes will be killed while other cells father away from the electrodes will not uptake the genetic material. Separating the electrodes by a greater distance permits greater flexibility in the device used to delivery the genetic material and also permits a larger target area of cells.

Additionally, the present invention enhances efficiency of cellular uptake. As a result, a larger percentage of the target cells uptake the genetic material. This increased uptake also enhances the desired biological reaction by cells in the target area such as increased cell expression of biological material or programmed cell death. Increasing efficiency of cellular uptake of the genetic material also increases the cost efficiency of the treatment because less genetic material is required in order to treat the patient.

There are related advantages if vectors are used to carry the genetic material. If viral vectors are used, reducing the amount of material that is delivered may decrease the risk and severity of an allergic reaction. Because lipid-based carriers of genetic material are expensive, the cost benefit of the present invention is still further enhanced when this form of genetic material is used. Yet another advantage is that the need to use vectors may be eliminated altogether so that naked genetic material can be delivered to the target cells. The use of naked genetic material further increases the simplicity and decreases the cost of administering therapies based on delivering genetic material.

The process of transferring genetic material as shown in Figured 1A-1D stands in sharp contrast to electroporation, which is illustrated in Figure 2. During electroporation, energy levels as high as 2 kV/cm to 8 kV/cm is applied to the cell 10. This high-level of energy blasts a hole 22 in the cellular membrane 16, and the genetic material 20 is then taken directly into the cytoplasm 18.

As described above, electroporation has significant disadvantages. For example, there is a narrow range of energy levels that can be applied to the target area and then the energy can be applied for only a very short duration. Target cells will be killed if the energy is too high. Likewise, target cells will not be porated if the energy level is too low, especially considering that the energy is

applied in short pulses. Therefore, the present invention has significant advantages over prior delivery techniques such as electroporation.

Referring to Figure 3, the present invention can be implemented using an apparatus 24 that has a hypodermic needle 26 that is in fluid communication with a reservoir 28 such as a syringe. The hypodermic needle 26 is formed from an electrically conductive material. A power supply 30 is electrically connected to the hypodermic needle 26 via a lead 32. The power supply 30 is also electrically connected to a patch-type electrode 34 via another lead 36. Although one particular delivery apparatus is disclosed, the present invention can be implemented using a variety of structures that have at least two electrodes of an opposite polarity and a mechanism for delivering the genetic material to the target site. The apparatus and method described herein are useful to treat a variety of cells with genetic material including cells within solid tumors, vital organs such as the liver and the heart, muscles, the vascular system, and infections deep seated within bone.

In use, the caregiver places the patch-type electrode 34 against the patient's skin in a convenient location such as the patient's thigh or abdomen. The caregiver injects the needle 26 into the site of the target cells. The syringe is used to inject the genetic material to a position proximal to the target cells, which in many instances is within the interstitial spaces between the target cells. The distance between the patch-type electrode and the needle is about 10 cm or more.

A voltage gradient is created between the needle 26 and the patch-type electrode 34, and a current flows through the target area of cells. In one possible embodiment, the apparatus and method described herein use low level of energy that is between about 0.1 mA and about 0.8 A. In one possible embodiment the current is below about 20 mA when direct current is used. In another possible embodiment that used direct current, the current is between about 0.1 mA and about 20 mA. In yet another possible embodiment, the current is below about 0.8 A when the current has a pulsed or alternating waveform. In this embodiment, the amplitude of the current depends on the pulse width and duty cycle; the shorter the pulse width, the higher the amplitude of the current that can be used. In another possible

embodiment that uses pulsed or alternating current, the amplitude is between about 0.2 A and about 0.8 A.

In one possible embodiment, the voltage gradient applied between the patch-type electrode and the needle is between about 5 mV/cm and about 200 V/cm. In one possible embodiment the current is below about 10 V/cm when direct current is used. In another possible embodiment that uses direct current, the current is between about 5 mV/cm and about 10 V/cm. In yet another possible embodiment, the current is below about 200 V/cm when the current has a pulsed or alternating waveform. In another possible embodiment that uses pulsed or alternating current, the amplitude is between about 10 V/cm and about 200 V/cm.

In embodiments that use a pulsed or alternating waveform, the amplitude of the current depends on the pulse width and duty cycle. The shorter the pulse width, the higher the amplitude of the current that can be used. In certain instances, the voltage level might be higher than 200 V/cm and still not porate the cells if the pulse width is sufficiently short.

These ranges energy are significantly lower than those used for electroporation. As discussed above, for example, electroporation typically uses voltage levels of about 2 kV/cm to about 8 kV/cm. Additionally, the electrodes are placed within a very close proximity to one another. In a typical embodiment, for example, the nominal distance between electrodes is 0.5 cm. A typical resistance for the circuit is about 500 Ω. Using ohm's law, then, the current used for electroporation typically ranges between about 2 A and 8 A. These dangerously high levels of current are as much as 80,000 times (which equals 8 A/0.1 mA) those used by the present invention.

A variety of mechanisms to deliver the genetic material to the target site can be used. Examples include iontophoresis, phonophoresis, injection, and the like. Furthermore, the low level of electrical energy can be applied to the target area before, during, or after delivery of the genetic material.

Additionally, the cardiac cycle of the patient's heart can be paced with an electrical pulse, and the voltage gradient and electrical current then can be synchronized with the cardiac cycle of the patient's heart in order to minimize the risk of inducing an arrhythmia. Pacing and synchronizing application of the

electrical energy is advantageous when the target cells are located in the myocardium of the heart. Simultaneous cardiac pacing and local drug delivery are described in more detail in United States Patent 5,634,899, which issued on June 3, 1997, the disclosure of which is hereby incorporated by reference. Furthermore, the voltage gradient and electrical current conducted between the electrodes can have a variety of waveforms. Such waveforms are also described in United States Patent 5,634,899.

Examples of treatments that rely on the delivery and cellular uptake of genetic material include the delivery of angiogenesis regulators, which are agents that either promote or inhibit the development of new vasculature; the administration of genetic vaccines; and the delivery of genetic material that promotes apoptosis, which is programmed cell death and is useful in treating certain maladies such as solid tu[illegible]ic fibrosis, which is caused when cells generate a defec[illegible] of restenosis, which is the reclosure of an artery [illegible]rms of gene therapy or delivery of antisense molecu[illegible]ors can be either positive or negative. Positive angiogenesis regulators promote the development of new vasculature and are useful to promote the generation of new vessels around a blockage in the cardiovascular system. Another possible application for positive angiogenesis agents is the generation of new vasculature in the legs of a person such as a diabetic that has poor circulation in his/her legs. Negative angiogenesis regulators are useful to prevent excessive proliferation of vasculature. Such excessive proliferation of blood vessels can promote the development and spreading of tumors, blindness, and arthritis.

Examples of positive angiogenesis regulators include fibroblast growth factors, vascular endothelial growth factors, placental growth factors, transforming growth factors, angiogenin, interleukin-8, hepatocyte growth factors, granulocyte colony-stimulating growth factors, and platelet-derived endothelial cell growth factors. Examples of negative angiogenesis growth factors include thrombospondin-1, angiostatin, alpha interferon, prolactin 16-kd fragments, metallo-proteinase inhibitors, platelet factor 4, placental proliferin-related protein, and transforming growth factor beta.

The use of low levels of electrical energy enhances the process of cellular ingestion of the genetic material as described in conjunction with Figures 1A-1D. This enhancement is demonstrated with several experimental examples as follows.

Example #1

Bovine aortic smooth muscle cells % confluency in 9 clear transwells. The cells were i ied Eagle's Medium (DMEM) plus 10% Fetal Bovine tic solution. The cells were exposed to the plasmid p oding sequence for the alkaline phosphatase gene. The concentration of 200 µg/ml in D5W for a total of 400µg per well. During exposure to the gene plasmid, a first well was treated with 1 mA of current for 1 minute and a second well was treated with 10 mA for 1 minute. A third, control well of cells was exposed to plasmid at an equal concentration with no current. The total time of exposure for the control well was designed to match that of the experimental groups (the handling time of the electrodes additional to the actual time of iontophoresis). Each condition was repeated in triplicate using three separate runs.

The bottom medium in each well contained the positive silver wire electrode in DMEM, pH 7.5. The top medium in each well contained the negative silver/carbon patch (AgCl) along with the DNA-D5W solution. After iontophoresis, the cells were fed with fresh DMEM plus 10% FBS plus IX antibiotic solution and incubated at 37°C for 24 hours. The cells were stained for alkaline phosphatase and counted.

The data and results of the experiment are as follows:

During Treatment

Current (mA)	Actual Time (min)
0	2:00
0	2:00
0	2:00
1.0	2:35
1.0	1:55
1.0	1:54
10.0	1:46
10.0	1:47
10.0	1:55

Cell Counts After 24 Hours Incubation

Current (mA)	Count of Transfected Cells per Well (n = 3)
0	mean=51.3±13.9
1.0	mean=896.7±60.1
10.0	mean=894.3±125.7

This experiment demonstrates a significant increase in overall transduction (17-fold) when using 1.0 mA of current over that of the control (passive treatment). There is also an increase of overall transduction when using 10.0 mA over that of the control. The increase in transduction using 10 mA of current is not higher than the increase in transduction using 1 mA of current. The monowell used in triplicate provides less sample error within a certain condition than the triwell system.

Example #2

Bovine aortic smooth muscle cells were plated at a density of 70% confluency in 6 clear transwells. The cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) plus 10% Fetal Bovine Serum (FBS) plus IX antibiotic solution. The cells were exposed to adenovirus containing the coding sequence for the Beta-galactosidase gene. The adenovirus was diluted to a concentration of 1 x 10^7 pfu/ml using D5W. A first well was exposed to 1 mA of current for 1 minute, and a second well was exposed to 10 mA of current for 1 minute. A third, control well was exposed to adenovirus for a time that reflects total exposure time in the experimental wells. The bottom medium in each well contained the positive silver wire electrode in DMEM. The top medium in each well contained the negative silver/carbon patch (AgCl) along with the virus/D5W solution. Each condition was performed in duplicate using separate runs. After iontophoresis, the cells were fed with fresh DMEM plus 10% FBS plus 1X antibiotic solution and incubated at 37°C for 24 hours. The cells were stained for beta-galactosidase, nuclear stained with DAPI and counted.

The data and results of the experiment are as follows:

During Treatment

Current (mA)	Time
0	1:50
0	1:49
1.0	1:51
1.0	1:45
10.0	1:54
10.0	1:52

Cell Counts After 24 Hours Incubation

Current (mA)	Transfected Cells per Well (n=10)	
0	mean=6.42%±2.7%	(n=15)
1.0	mean=11.5%±3.3%	(n=19)
10.0	mean=42.9%±15.2%	(n=14)

This experiment demonstrates that there is a significant increase in transduction efficiency (6-fold) when using 10.0 mA of current compared to the control.

The various embodiments described above are provided by way of illustration only and should not be construed to limit the invention. Those skilled in the art will readily recognize various modifications and changes that may be made to the present invention without following the example embodiments and applications illustrated and described herein, and without departing from the true spirit and scope of the present invention, which is set forth in the following claims.

The claimed invention is:

1. A method for delivering genetic material into a plurality of target cells, the method comprising the steps of:

delivering genetic material to a position proximal the plurality of target cells; and

subjecting the plurality of target cells to electrical energy at a level of about 0.8 A or below, thereby mediating cellular expression.

2. The method of claim 1 wherein constant, continuous direct current is used and the current level is between about 0.1 mA and about 20 mA.

3. The method of claim 1 wherein the current has a pulsed wave form and the current level is between about 0.1 mA and about 0.8 A.

4. The method of claim 1 wherein the genetic material is in a form selected from the group consisting essentially of naked DNA; naked RNA; gene plasmids; antisense oligonucleotides; ribozymes; viral vectors including adenoviruses, retroviruses, adeno-associated viruses, and viral envelop/capsid-DNA complexes; DNA-protein complexes; DNA in complexes with natural or synthetic polymers; lipid-based compositions; and liposome-based compositions.

5. The method of claim 1 wherein the genetic material encodes an angiogenic growth factor.

6. The method of claim 5 wherein the angiogenic growth factor is a positive regulator and selected from the group consisting essentially of fibroblast growth factors, vascular endothelial growth factors, placental growth factors, transforming growth factors, angiogenin, interleukin-8, hepatocyte growth factors, granulocyte colony-stimulating growth factors, and platelet-derived endothelial cell growth factors.

7. The method of claim 1 wherein the genetic material encodes a negative angiogenic regulator.

8. The method of claim 1 wherein the genetic material comprises a genetic vaccine.

9. The method of claim 1 wherein the plurality of target cells are in a patient and the step of delivering the genetic material includes the steps of:

providing a reservoir that includes the genetic material; and

transporting the genetic material from the reservoir to the position proximal the plurality of target cells within the patient.

10. The method of claim 9 wherein the patient has a heart and the plurality of target cells are cells in the myocardium of the heart, the step of delivering the genetic material includes the step of transporting the genetic material into the myocardium of the heart.

11. The method of claim 9 wherein the patient has a leg and the plurality of target cells are cells in the leg, the step of delivering the genetic material includes the step of transporting the genetic material into the leg.

12. The method of claim 9 wherein the patient has an extremity and the plurality of target cells are cells in the extremity, the step of delivering the genetic material includes the step of transporting the genetic material into the extremity.

13. The method of claim 1 wherein the plurality of target cells are within a target area and the step of subjecting the plurality of target cells to electrical energy includes the step of conducting an electrical current through the target area, the current being about .4 A or less.

14. The method of claim 13 wherein the step of subjecting the plurality of target cells to electrical energy includes the additional steps of:

placing first and second electrodes into electrical contact with the plurality of target cells;

supplying a pacing pulse between the first and second electrodes initiating at least one cardiac cycle; and

synchronizing conduction of electrical current to the pacing pulse in a predetermined manner within the remaining portion of the cardiac cycle to reduce the risk of inducing arrhythmia.

15. The method of claim 14 wherein the current is a direct current.

16. The method of claim 14 wherein the current is an alternating current.

17. The method of claim 14 wherein the current has a waveform that provides a net positive voltage.

18. The method of claim 14 wherein the target cells comprise a myocardial cell.

19. The method of claim 13 wherein the step of subjecting the plurality of target cells to electrical energy includes the additional steps of:

placing first and second electrodes into electrical contact with the plurality of target cells;

sensing intrinsic electrical activity of a heart for at least one cardiac cycle; and

synchronizing conduction of electrical current to the intrinsic electrical activity of the heart in a predetermined manner within the remaining portion of the cardiac cycle to reduce the risk of inducing arrhythmia.

20. The method of claim 19 wherein the current is a direct current.

21. The method of claim 19 wherein the current is an alternating current.

22. The method of claim 19 wherein the current has a waveform that provides a net positive voltage.

23. The method of claim 19 wherein the target cells comprise a myocardial cell.

24. The method of claim 13 wherein the step of subjecting the plurality of target cells to electrical energy includes the steps of:

placing first and second electrodes into electrical communication with the target area; and

creating a voltage gradient between the first and second electrodes, the voltage gradient being about 200V/cm or less.

25. The method of claim 1 wherein the step of subjecting the plurality of target cells to electrical energy includes the step of placing first and second electrodes into electrical contact with the plurality of target cells.

26. The method of claim 25 wherein the first and second electrodes are spaced by more than about 0.3 cm.

27. The method of claim 25 wherein:

the step of delivering genetic material includes the step of providing a reservoir that includes genetic material; and

the step of placing first and second electrodes into electrical contact with the plurality of target cells includes the step of placing the genetic material within the electrical path between the first and second electrodes.

28. The method of claim 27 wherein the plurality of target cells forms a target area, the method comprising the additional step of iontophoretically transporting the genetic material from the reservoir to the target area.

29. The method of claim 28 wherein the step of iontophoretically transporting the genetic material includes conducting a positive net flow of current in one direction between the first and second electrodes.

30. The method of claim 29 wherein the step of subjecting the plurality of target cells to electrical energy includes the step of conducting an alternating current through the target area, the alternating current having a net current of about zero.

31. The method of claim 1 wherein the plurality of target cells are within a patient and the step of delivering genetic material includes the step of injecting the genetic material into the patient.

32. The method of claim 31 wherein the step of subjecting the plurality of target cells to electrical energy is performed before the step of injecting the genetic material.

33. The method of claim 31 wherein the step of subjecting the plurality of target cells to electrical energy is performed after the step of injecting the genetic material.

34. The method of claim 31 wherein the step of subjecting the plurality of target cells to electrical energy is performed simultaneously with the step of injecting the genetic material.

35. An apparatus for delivering genetic material into a target area in a patient's body and mediating cellular expression of genes in the target area, the apparatus comprising:

a reservoir arrangement, the reservoir arrangement being configured to retain genetic material and supply the genetic material to the target area;

first and second electrodes, the first electrode configured and arranged to be in electrical communication with the reservoir and the second electrode configured to be placed into electrical communication with the target area; and

a power supply in electrical communication with the first and second electrodes, the power supply configured to pass energy between the first and second electrodes at a level of about 0.8 A or below.

36. The apparatus of claim 35 wherein the power supply is configured to provide a voltage gradient between the first and second electrodes of about 200V/cm or less.

37. The apparatus of claim 35 wherein the reservoir is loaded with genetic material.

38. The apparatus of claim 35 wherein the first electrode is connected to the reservoir arrangement.

39. The apparatus of claim 38 wherein the second electrode is a patch-type electrode.

40. The apparatus of claim 38 wherein the first electrode is needle formed of conductive material.

41. An apparatus for delivering genetic material into a target area in a patient's body and mediating cellular expression of genes in the target area, the apparatus comprising:

means for retaining a supply of genetic material;

means for transporting genetic material from the means for retaining a supply of genetic material to the target area; and

means for supplying electrical energy of about 0.4 A or below to the target area thereby enhancing uptake of the genetic material into cells in the target area.

FIG.1A

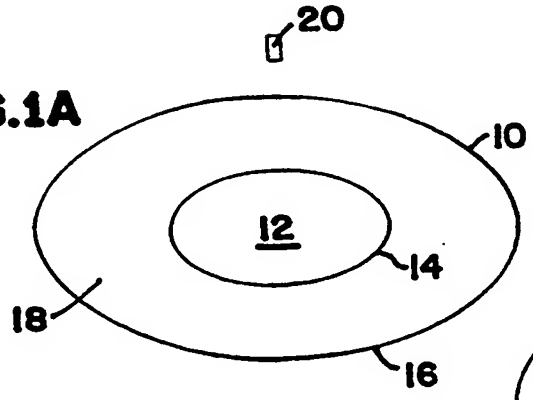


FIG.1B

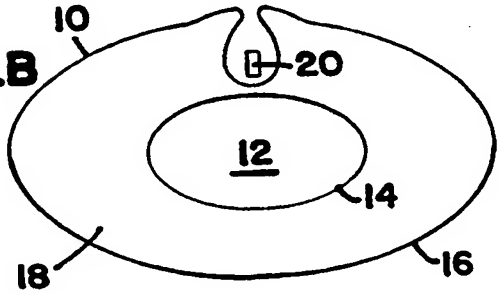


FIG.2

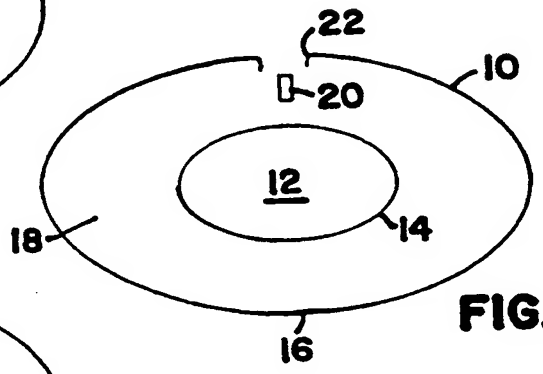


FIG.1C

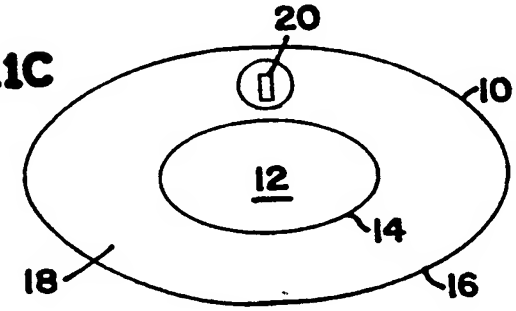
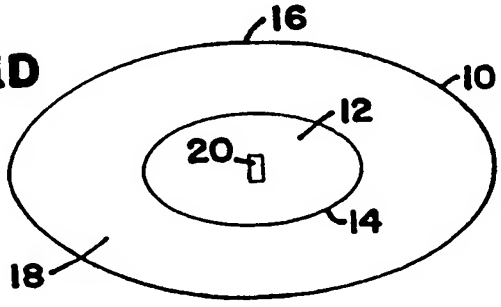


FIG.1D



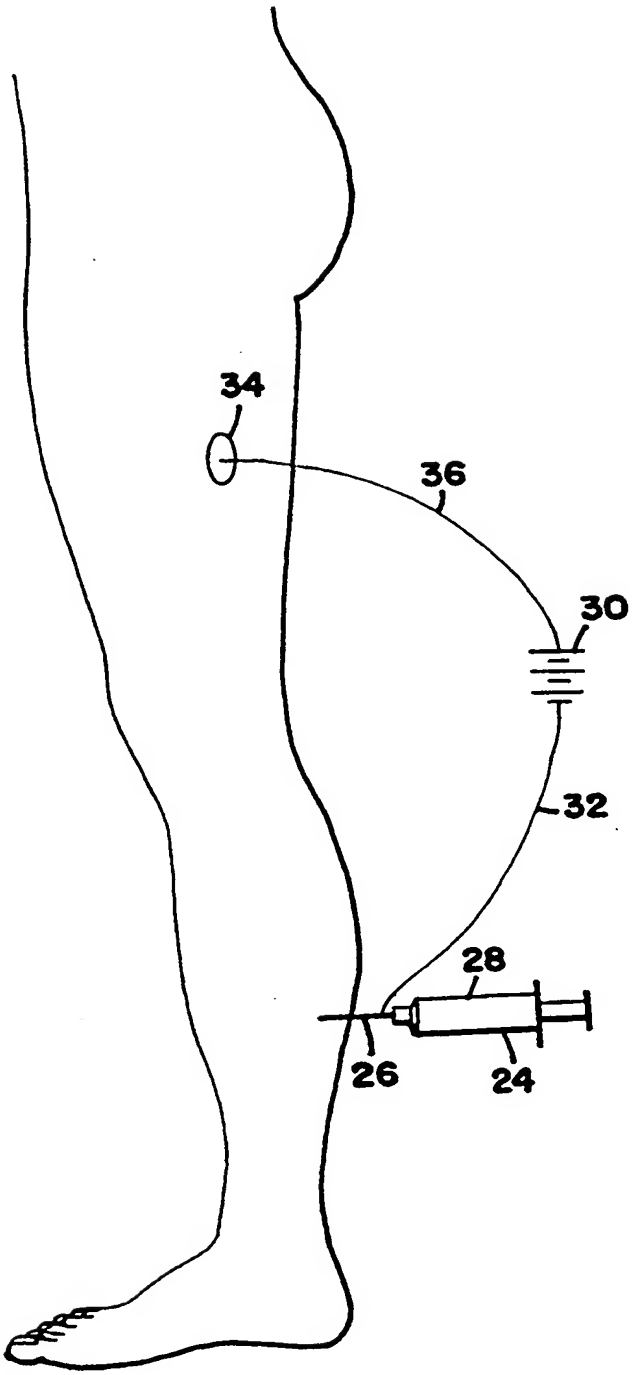


FIG.3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/00824

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/87 A61N1/30 A61N1/32 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39531 A (MASSACHUSETTS INST TECHNOLOGY) 12 December 1996 abstract, page 2,3; page 4,5,6,7; page 10, line 26; page 12, line 10; ---	1-4,8,9, 11-13, 24-29, 35-38,41
X	GB 2 200 367 A (INST BOTAN IM N G KHOLODNOGO A) 3 August 1988 abstract; page 1, line 20-24; page 2; example 4, claims --- -/--	1,3,4, 13, 24-28, 35-38,41

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 May 1999	25/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/00824

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GEEST VAN DER R ET AL: "IONTOPHORESIS: A TRANSDERMAL DELIVERY STRATEGY FOR ANTISENSE OLIGONUCLEOTIDE DRUGS ?" PHARMACEUTICAL RESEARCH, vol. 9, no. 10, 1 October 1992, page S-67 XP000655154 see the whole document	1,2,4,9, 12,13, 24-28, 33-41
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A	WO 95 05853 A (UNIV CALIFORNIA ;CARSON DENNIS A (US); RAZ EYAL (US); HOWELL MERED) 2 March 1995 abstract; page 11,12,41; claim 9	1-41
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E,L	WO 99 04850 A (EMED CORP) 4 February 1999 abtract; page 1,3; page 5, line 3; page 9,10; claims; Figures; L. Priority	1-9,12, 13,18, 24-41

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